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L9: Entry 1 of 1

File: USPT

Mar 9, 1999

DOCUMENT-IDENTIFIER: US 5880146 A

TITLE: Inhibition of IL-12-induced IFN- γ synthesis by specific bis-phenol compounds as a method of immune modulationDetailed Description Paragraph Right (21):

Human peripheral blood monocytes (PBMC) were obtained from commercial sources as a leukophoresis from a healthy volunteer and were purified by centrifugation on a Ficoll-Hypaque (Pharmacia) gradient (1700 rpm for 20 min). The "buffy" coat containing the PBMC was diluted with serum-free culture medium (SF-RPMI) to a volume of 50 ml and collected by centrifugation at 1500 rpm for 5 min. Cells were resuspended in cell culture medium containing 10% fetal bovine serum (RPMI-10) and phytohemagglutinin (PHA - 10 $\mu\text{g/ml}$) at a density of 5×10^6 cells/ml and were cultured for 3 days at 37 $^\circ\text{C}$ in a humidified CO $_2$ incubator. The PHA-activated cells were collected by centrifugation, washed three times with an equal volume of SF-RPMI and resuspended in fresh RPMI-10 (1×10^6 cells/ml). Aliquots (100 μl) were dispensed into the wells of multiple 96-well plates to give a final cell number of 10^5 per well. Test compounds, dissolved in dimethyl sulfoxide (DMSO) at 1 mg/ml, were first diluted in culture medium to an intermediate concentration of 20 $\mu\text{g/ml}$ and then were added (50 $\mu\text{l/well}$) to a specific well of the plate for each compound. Stimulation medium (50 $\mu\text{l/well}$) containing 10% serum, IL-2 and IL-12 was added to final concentrations of 25 U/ml and 0.5 ng/ml, respectively. Control wells receive no IL-2 or IL-12 (negative control) or received both interleukins but no test compound (positive control). The plates were incubated for 48 hr at 37 $^\circ\text{C}$ in a CO $_2$ incubator at which time aliquots (20 μl) were removed for analysis of IFN- γ concentration by ELISA. A quantitative ELISA was developed by coating 96-well plates with an mouse monoclonal antibody against human IFN- γ , 1 $\mu\text{g/ml}$ in phosphate buffered saline (PBS) (Pestka Biological Laboratories), overnight at 4 $^\circ\text{C}$. Unbound antibody was washed off by washing three times with PBS. Non-specific antibody binding was blocked with a solution of 1% bovine serum albumin (BSA) and 1% goat serum in PBS (150 $\mu\text{l/well}$) which was incubated for 2 hr at 37 $^\circ\text{C}$. After washing the blocked plates four times with PBS, test samples and dilutions of the IFN- γ standard are added in a final volume of 100 $\mu\text{l/well}$. Following an overnight incubation at 4 $^\circ\text{C}$, the plates are washed four times with PBS, and a polyclonal rabbit antiserum against human IFN- γ (1/10000 dilution--Pestka Biological Laboratories) is added. After an additional incubation for 1 hr at 37 $^\circ\text{C}$ and four washes with PBS, a polyclonal donkey anti-rabbit detecting antibody, conjugated to horseradish peroxidase (1/700 dilution--Pestka Biological Laboratories) is added for 1 hr at 37 $^\circ\text{C}$. The plates are then washed four times with PBS and 100 μl of K-blue substrate E (ELISA Technologies, Neogen Corp.) is added until the color in the wells containing the standard curve is sufficiently developed, at which time 100 μl of "Red-stop" solution (ELISA Technologies) is added. The absorbance of the solution within each well of the plate is then read at 650 nm using an ELISA plate reader (Dynatech MR7000). The amount of IFN- γ is calculated by comparing the optical density of the test sample with a standard curve derived from the dilutions of the control IFN- γ . The amount of IFN- γ that is induced in the presence of both IL-2 and IL-12 generally ranges from 1200-2000 pg/ml while the amount produced in the absence of IL-12 is generally less than 50 pg/ml. Experimental data are shown in Table 2 which discloses a) the percent inhibition of IFN- γ production, relative to a negative control of untreated cells, when the

cells have been treated with a bis-compound at a final concentration of 5 .mu.g/ml of the listed bis-compound (%INH or IFN.gamma.) and b) the percent of cells which are viable after the treatment with 5 .mu.g/ml of the various bis-compounds, determined by adding MTS to the media (% Viability). MTS is a chemical chromophore that is metabolized in the mitochondria of viable cells to produce a color which increases in intensity in proportion to the numbers of viable cells. The absorbance of the cell culture can be compared to control cultures in order to determine the percent viability.

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L13: Entry 8 of 8

File: USPT

Jul 30, 1996

DOCUMENT-IDENTIFIER: US 5541310 A

TITLE: Herbicide resistant plants

Detailed Description Paragraph Right (5):

Increased expression of herbicide-sensitive IGPD also can be accomplished by stably transforming a plant cell with a recombinant or chimeric DNA molecule comprising a promoter capable of driving expression of an associated structural gene in a plant cell, linked to a homologous or heterologous structural gene encoding IGPD. By "homologous," it is meant that the IGPD gene is isolated from an organism taxonomically identical to the target plant cell. By "heterologous," it is meant that the IGPD gene is obtained from an organism taxonomically distinct from the target plant cell. IGPD genes can be obtained by complementing a bacterial or yeast auxotrophic mutant with a plant cDNA library. See, e.g. Snustad et al, Genetics 120:1111-1114 (1988) (maize glutamine synthase); Delauney et al., Mol. Genet. 221:299-305 (1990) (soybean-pyrroline-5-carboxylate reductase); Frisch et al., Mol. Gen. Genet. 228:287-293(1991) (maize dihydrodipicolinate synthase); Eller et al., Plant Mol. Biol. 18:557-566 (1992) (rape chloroplast 3-isopropylmalate dehydrogenase); Proc. Natl. Acad. Sci, USA 88:1731-1735 (1991); Minet et al., Plant J. 2:417-422 (1992) (dihydroorotate dehydrogenase) and references cited therein. Other known methods include screening genomic or cDNA libraries of higher plants, for example, for sequences that cross-hybridize with specific nucleic acid probes, or by screening expression libraries for the production of IGPD enzymes that cross-react with specific antibody probes. A preferred method involves complementing an E. coli his B auxotrophic mutant with an Arabidopsis thaliana cDNA library.

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L10: Entry 5 of 32

File: USPT

Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287790 B1

TITLE: Utilization of nuclear structural proteins for targeted therapy and detection of proliferative and differentiation disorders

Detailed Description Paragraph Right (68):

Suitable antibodies specific to NuMA may be made by conventional monoclonal or polyclonal techniques. Based on our work and other data present in the literature, it is possible to identify cells in mitosis, proliferating, growth-arrested, differentiated and dying, according to the pattern of NuMA distribution (see scheme: FIG. 14). NuMA staining will enable the identification of proliferating cells, as shown by a diffuse pattern within the nucleus, and mitosis, as shown by the localization of NuMA to the pole of the mitotic spindle. It is also possible to recognize growth-arrested cells, as shown by the patchier distribution of NuMA within the nucleus, although the use of the punctateness algorithm may be easier to apply here for non trained individuals. Cells undergoing cell death by apoptosis show a NuMA staining concentrated in one large foci in the center of the nucleus. Finally, cellular differentiation resembling the state of differentiation observed in mammary tissues can be recognized by the formation of enlarged and peripheral NuMA foci within the nucleus. The latter may be restricted to mammary epithelial cells until further analysis of other cell types, while the other distribution patterns may be used in many different cell and tissue types since NuMA as been shown to be present in numerous cell lines. Identification of the various types of NuMA distribution could be used for instance in the course of an experiment in cell culture or to assess the number of actively proliferating cells or dying cells in a tissue. This could be done to compare control and drug-treated cells or tissues, and in any other studies which involve alteration of the cellular behavior. The use of NuMA to discriminate between different cell behaviors could be applied for both non-malignant and malignant cells.

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L3: Entry 1 of 4

File: USPT

Feb 5, 2002

DOCUMENT-IDENTIFIER: US 6344322 B1

TITLE: Subtle mitochondrial mutations as tumor markers

Detailed Description Paragraph Right (6):

Mutations can first be identified by comparison to sequences present in public databases for human mitochondrial DNA, e.g., at <http://www.gen.emory.edu/mitomap.html>. Any single basepair substitution identified in the sample DNA compared to a normal sequence from a database can be confirmed as being a somatic mutation as opposed to a polymorphic variant by comparing the sample mitochondrial DNA or sequences obtained from it to control cell mitochondrial DNA from the same individual or sequences obtained from it. Control cells are isolated from other apparently normal tissues, i.e., tissues which are phenotypically normal and devoid of any visible, histological, or immunological characteristics of cancer tissue. A difference between the sample and the control identifies a somatic mutation which is associated with the tumor.

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<u>L11</u>	L8 same chloroplast	0	<u>L11</u>
<u>L10</u>	L8 same nucleus	32	<u>L10</u>
<u>L9</u>	L8 same mitochondria	1	<u>L9</u>
<u>L8</u>	L7 same compar\$ same (standard\$ or control\$)	5329	<u>L8</u>
<u>L7</u>	(screen\$ or detect\$ or identif\$ or monitor\$) same (drug\$ or agent\$ or compound\$)	129626	<u>L7</u>
<u>L6</u>	L2 same chloroplast	0	<u>L6</u>
<u>L5</u>	L2 same nucleus	4	<u>L5</u>
<u>L4</u>	L2 same nucle	0	<u>L4</u>
<u>L3</u>	L2 same mitochondri\$	4	<u>L3</u>
<u>L2</u>	L1 same compar\$ same (standard\$ or control\$)	967	<u>L2</u>
<u>L1</u>	(screen\$ or detect\$ or identif\$ or monitor\$) same cancer\$	14605	<u>L1</u>

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L10: Entry 4 of 32

File: USPT

Oct 2, 2001

DOCUMENT-IDENTIFIER: US 6297253 B1

TITLE: Compounds and methods of use to treat infectious diseases

Detailed Description Paragraph Right (65):

The assays are performed in vitro in which the cellular receptor moiety is immobilized directly or indirectly onto a solid support. The NLS-containing protein or molecular complex, purified or in a cell extract, is contacted with the immobilized cellular receptor moiety in the presence of test compound. As a control, the NLS-containing protein or molecular complex is contacted with the immobilized cellular receptor moiety under the same condition, but in the absence of the test compound. After an interval sufficient for binding reactions to occur among the components in the assay, the solid support is washed to remove any unbound molecules. A detection procedure is performed with the solid support to quantify the binding of NLS-containing protein or molecular complex to the immobilized cellular receptor moiety as compared to binding reactions in the absence of test compound. The absence of bound NLS-containing protein or molecular complex, or a reduction in the binding of the NLS-containing protein or molecular complex to the solid support, in the presence of a test compound, indicates that the test compound can be useful in inhibiting the importation of the specific NLS-containing protein or molecular complex into the nucleus.

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L5: Entry 1 of 4

File: USPT

Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277844 B1

TITLE: Compound for selective treatment of malignant cells by inhibiting cell cycle progression, decreasing Bcl2, and increasing apoptosis

Detailed Description Paragraph Right (53):

In the method of the present invention, compounds are screened for use as chemotherapeutic agents based upon their competitive inhibition of binding of BBL22 within the cell. In a preferred screening method, cell cultures are established using cell lines exemplified by MCF7 (a human mammary carcinoma cell line) and MDA-MB-468 (a breast cancer cell line). Fluorescent-labeled BBL22 is added to cell cultures in conjunction with an excess of target compound. A potentially useful target compound is identified as a compound which inhibits the binding of BBL22 to its target site, thereby causing BBL22 to remain unbound. Inhibition of BBL22 is indicated by the absence of fluorescent staining to nuclei of malignant cells treated with BBL22 and a target compound, as compared to fluorescence stained nuclei of malignant control cells treated with BBL22 alone. This technique may also be used in high-throughput screening using microtiter plates.

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